AMENDMENTS TO THE SPECIFICATION:

Pursuant to 37 C.F.R. § 1.121, please amend the specification as follows. Amendments comprising additions to the specification are shown by bold **double-underline**. Amendments comprising deletions to the specification are shown by bold ~~**strikethrough**~~.

Please replace the paragraph beginning at page 1, line 7, with the following amended paragraph:

This application is a **continuation of and claims priority to U.S. Patent Application Serial No. 08/769,062, filed December 18, 1996, now U.S. Patent No. 6,335,160,** ~~**continuation-in-part of U.S. Patent Application Serial No. 08/198,431, filed February 17, 1994, Serial No. PCT/US95/02126, filed, February 17, 1995, Serial No. 08/425,684, filed April 18, 1995, Serial No. 08/537,874, filed October 30, 1995, Serial No. 08/564,955, filed November 30, 1995, Serial No. 08/621,859, filed March 25, 1996, Serial No. 08/621,430, filed March 25, 1996, Serial No. PCT/US96/05480, filed April 18, 1996, Serial No. 08/650,400, filed May 20, 1996, Serial No. 08/675,502, filed July 3, 1996, Serial No. 08/721,824, filed September 27, 1996, and 08/722,660 filed September 27, 1996**~~ the **specification** ~~**specifications**~~ of which **is** ~~**are**~~ herein incorporated by reference in **its** ~~**their**~~ entirety for all purposes.

Please replace the paragraph beginning at page 11, line 17 with the following amended paragraph:

A further aspect of the invention is a method for evolving the coupling of a mammalian 7-transmembrane receptor to a yeast signal transduction pathway, comprising:

(a) expressing a library of mammalian G alpha protein mutants in a host cell, wherein the host cell expresses the mammalian 7-transmembrane receptor and a reporter gene, the receptor gene **being** ~~**geing**~~ expressed under control of a pheromone responsive promoter;

(b) screening or selecting the products of (a) for expression of the reporter gene in the presence of a ligand for the 7-transmembrance receptor; and

(c) recovering DNA encoding an evolved G alpha protein mutant from screened or selected products of (b).

Please replace the paragraph beginning at page 12, line 22 with the following amended paragraph:

Figures 2A-2G depict ~~Figure 2 depicts~~ the alignment of alpha interferon amino acid and nucleic acid sequences **(SEQ ID NOS:75-86 and 87-98, respectively)**.

Please replace the paragraph beginning at page 13, line 5 with the following amended paragraph:

Coarse grain and fine grain shuffling allow analysis of variation **occurring** ~~occuring~~ within a nucleic acid sequence, also termed "searching of sequence space." Although both techniques are meritorious, the results are qualitatively different. For example, coarse grain searches are often better suited for optimizing multigene clusters such as polyketide operons, whereas fine grain searches are often optimal for optimizing a property such as protein expression using codon usage libraries.

Please replace the paragraph beginning at page 14, line 1 with the following amended paragraph:

Some formats and examples for recursive sequence recombination, sometimes referred to as DNA shuffling, evolution, or molecular breeding, have been described by the present inventors and co-workers in co-pending applications U.S. Patent Application Serial No. 08/198,431, filed February 17, 1994, Serial No. PCT/US95/02126, filed, February 17, 1995, Serial No. 08/425,684, filed April 18, 1995, Serial No. 08/537,874, filed October 30, 1995, Serial No. 08/564,955, filed November 30, 1995, Serial No. 08/621,859, filed March 25, 1996, Serial No. 08/621,430, filed March 25, 1996, Serial No. PCT/US96/05480, filed April 18, 1996, Serial No. 08/650,400, filed May 20, 1996, Serial No. 08/675,502, filed July 3, 1996, Serial No. 08/721, 824, filed September 27, 1996, and 08/722,660 filed September 27, 1996; Stemmer, Science 270:1510 (1995); Stemmer et al., Gene 164:49-53 (1995); Stemmer, Bio/Technology 13:549-553 (1995); Stemmer, Proc. Natl. Acad. Sci. U.S.A. 91:10747-10751 (1994); Stemmer, Nature 370:389-391 (1994); Crameri et al., Nature Medicine 2(1):~~1-3~~ **100-102** (1996); Crameri et al., Nature Biotechnology 14:315-319 (1996), each of which is incorporated by reference in its entirety for all purposes.

Please replace the paragraph beginning at page 16, line 18 with the following amended paragraph:

The starting DNA segments are recombined by any of the recursive sequence recombination formats **provided** ~~provded~~ herein to generate a diverse library of recombinant DNA segments. Such a library can vary widely in size from having fewer than 10 to more than 10^5, 10^9, or 10^{12} members. In general, the starting segments and the recombinant libraries generated include full-length coding sequences and any essential regulatory sequences, such as a promoter and polyadenylation sequence, required for expression. However, if this is not the case, the recombinant DNA segments in the library can be inserted into a common vector providing the missing sequences before performing screening/selection.

Please replace the paragraph beginning at page 22, line 9 with the following amended paragraph:

Having introduced the plasmids into cells, recombination between substrates to generate recombinant genes occurs within cells containing multiple different plasmids merely by propagating the cells. However, cells that receive only one plasmid are unable to participate in recombination and the potential contribution of substrates on such plasmids to evolution is not fully exploited (although these plasmids may contribute to some extent if they are **propagated** ~~progagated~~ in mutator cells). The rate of evolution can be increased by allowing all substrates to participate in recombination. Such can be achieved by subjecting transfected cells to electroporation. The conditions for electroporation are the same as those conventionally used for introducing exogenous DNA into cells (e.g., 1,000-2,500 volts, 400 μF and a 1-2 mM gap). Under these conditions, plasmids are exchanged between cells allowing all substrates to participate in recombination. In addition the products of recombination can undergo further rounds of recombination with each other or with the original substrate. The rate of evolution can also be increased by use of conjugative transfer. To exploit conjugative transfer, substrates can be cloned into plasmids having MOB genes, and *tra* genes are also provided in *cis* or in *trans* to the MOB genes. The effect of conjugative transfer is very similar to electroporation in that it allows plasmids to move between cells and allows recombination between any substrate and the products of previous recombination to occur, merely by propagating the culture. The rate of evolution can also be increased by fusing cells to induce exchange of plasmids or chromosomes. Fusion can be induced by chemical agents, such as PEG, or viral proteins, such as influenza virus hemagglutinin, HSV-1

gB and gD. The rate of evolution can also be increased by use of mutator host cells (e.g., Mut L, S, D, T, H in bacteria and Ataxia telangiectasia human cell lines).

Please replace the paragraph beginning at page 24, line 4 with the following amended paragraph:

After the second round of recombination, a second round of screening/selection is performed, preferably under conditions of increased stringency. If desired, further rounds of recombination and selection/screening can be performed using the same strategy as for the second round. With successive rounds of recombination and selection/ screening, the surviving recombined substrates evolve toward acquisition of a desired phenotype. Typically, in this and other methods of recursive recombination, the final product of recombination that has acquired the desired phenotype differs from starting substrates at 0.1%-25% of positions and has evolved at a rate orders of magnitude in excess (e.g., by at least 10-fold, 100-fold, 1000-fold, or 10,000 fold) of the rate of evolution driven by naturally acquired mutation of about 1 mutation per 10^{-9} positions per generation (see **<u>Andersson</u>** **~~Anderson~~** et al., <u>Proc. Natl. Acad. Sci. U.S.A</u>. 93:906-907 (1996)). The "final product" may be transferred to another host more desirable for utilization of the "shuffled" DNA. This is particularly advantageous in situations where the more desirable host is less efficient as a host for the many cycles of mutation/ recombination due to the lack of molecular biology or genetic tools available for other organisms such as *E. coli*.

Please replace the paragraph beginning at page 33, line 14 with the following amended paragraph:

In a further embodiment, the PCR primers for amplification of segments of the nucleic acid sequence of interest are used to introduce variation into the gene of interest as follows. Mutations at sites of interest in a nucleic acid sequence are identified by screening or selection, by sequencing homologues of the nucleic acid sequence, and so on. Oligonucleotide PCR primers are then synthesized which encode wild type or mutant information at sites of interest. These primers are then used in PCR mutagenesis to generate libraries of full length genes encoding permutations of wild type and mutant information at the designated positions. This technique is typically **<u>advantageous</u>** **~~advantagous~~** in cases where the screening or selection process is expensive,

cumbersome, or impractical relative to the cost of sequencing the genes of mutants of interest and synthesizing mutagenic oligonucleotides.

Please replace the paragraph beginning at page 34, line 26 with the following amended paragraph:

The amount of screening required to identify recombinants having two or more mutations can be dramatically reduced by the following technique. The nucleic acid sequences of interest are obtained from a pool of mutant clones and prepared as fragments, typically by digestion with a restriction endonuclease , sonication, or by PCR amplification. The fragments are denatured, then allowed to reanneal, thereby generating mismatched hybrids where one strand of a mutant has hybridized with a complementary strand from a different mutant or wild-type clone. The reannealed products are then fragmented into fragments of about 20-100 bp, for example, by the use of DNAseI. This fragmentation reaction has the effect of segregating regions of the template containing mismatches (mutant information) from those encoding wild type sequence. The mismatched hybrids can then be affinity purified using aptamers, dyes, or other agents which bind to mismatched DNA. A preferred embodiment is the use of mutS protein affinity matrix (Wagner et al., Nucleic Acids Res. 23(19):3944-3948 (1995); Su et al., Proc. Natl. Acad. Sci. (U.S.A.), 83:5057-5061(1986)) with a preferred step of amplifying the affinity-purified material in vitro prior to an assembly reaction. This amplified material is then put into a assembly PCR reaction as **described** ~~**decribed**~~ above. Optionally, this material can be titrated against the original mutant pool (e.g., from about 100% to 10% of the mutS enriched pool) to control the average number of mutations per clone in the next round of recombination.

Please replace the paragraph beginning at page 36, line 9 with the following amended paragraph:

In this method, a nucleic acid sequence, such as a gene or gene family, is arbitrarily defined to have segments. The segments are preferably exons. Introns are engineered between the segments. Preferably, the intron inserted between the first and second segments is at least about 10% divergent from the intron inserted between second and third segments, the intron inserted between second and third segments is at least about 10% divergent from the introns inserted between any of the previous segment pairs, and so on through segments n and n+1. The introns between any

given set of exons will thus initially be identical between all clones in the library, whereas the exons can be arbitrarily divergent in sequence. The introns therefore provide homologous DNA sequences that will permit application of any of the described methods for RSR while the exons can be arbitrarily small or divergent in sequence, and can evolve to achieve an arbitrarily large degree of sequence divergence without a significant loss in efficiency in recombination. Restriction sites can also be engineered into the intronic nucleic acid sequence of interest so as to allow a directed **reassembly** ~~**reassemmbly**~~ of restriction fragments. The starting exon DNA may be synthesized de novo from sequence information, or may be present in any nucleic acid preparation (e.g., genomic, cDNA, libraries, and so on). For example, 1 to 10 nonhomologous introns can be designed to direct recombination of the nucleic acid sequences of interest by placing them between exons. The sequence of the introns can be all or partly obtained from known intron sequence. Preferably, the introns are self-splicing. Ordered sets of introns and exon libraries are assembled into functional genes by standard methods (Sambrook et al., Molecular Cloning, CSH Press (1987)).

Please replace the paragraph beginning at page 37, line 15 with the following amended paragraph:

An example of how the introduction of an intron into a mammalian library format would be used advantageously is as follows. An intron containing a lox (Sauer et al., Proc. Natl. Acad. Sci. (U.S.A.), 85:5166-5170 (1988)) site is arbitrarily introduced between amino acids 92 and 93 in each alpha interferon parental substrate. A library of 10^4 chimeric interferon genes is made for each of the two exons (residues 1-92 and residues 93-167), cloned into a replicating plasmid vector, and introduced into target cells. The number 10^4 is arbitrarily chosen for convenience in screening. An exemplary vector for expression in mammalian cells would contain an SV40 origin, with the host cells expressing SV40 large T antigen, so as to allow transient expression of the interferon constructs. The cells are challenged with a cytopathic virus such as vesicular stomatitis virus (VSV) in an interferon protection assay (e.g., Meister et al., J. Gen. Virol. 67:1633-1643, (1986)). Cells surviving due to expression of interferon are recovered, the two libraries of interferon genes are PCR amplified, and recloned into a vector that can be amplified in *E. coli*. The amplified plasmids are then transfected at high multiplicity (e.g. 10 micrograms of plasmid per 10^6 cells) into a cre expressing host that can support replication of that vector. The presence of cre in the host cells promotes efficient recombination at the lox site in the interferon intron, thus shuffling the selected

sets of exons. This population of cells is then used in a second round of selection by viral challenge and the process is applied recursively. In this format, the cre recombinase is **preferably** ~~preferrably~~ expressed transiently on a cotransfected molecule that cannot replicate in the host. Thus, after segregation of recombinants from the cre expressing plasmid, no further recombination will occur and selection can be performed on genetically stable exon permutations. The method can be used with more than one intron, with recombination enhancing sequences other than cre/lox (e.g., int/xis, etc.), and with other vector systems such as but not limited to retroviruses, adenovirus or adeno-associated virus.

Please replace the paragraph beginning at page 40, line 35 with the following amended paragraph:

For example, this format is preferred for the in vivo affinity maturation of an antibody by RSR. In brief, a library of mutant antibodies is generated, as described herein for the 48G7 affinity maturation. This library is FACS purified with ligand to enrich for antibodies with the highest 0.1 - 10% affinity. The V regions genes are recovered by PCR, fragmented, and cotransfected or **electroporated** ~~electorporated~~ with a vector into which reassembled V region genes can recombine. DNA substrate molecules are recovered from the **cotransfected** ~~cotranfected~~ cells, and the process is repeated until the desired level of **improvement** ~~improvement~~ is obtained. Other embodiments include reassembling the V regions prior to the electroporation so that an intact V region exon can recombine into an antibody expression cassette. Further embodiments include the use of this format for other eukaryotic genes or for the evolution of whole viruses.

Please replace the paragraph beginning at page 41, line 16 with the following amended paragraph:

In some embodiments of the invention, a gene of interest is cloned into a vector that generates single stranded DNA, such as a phagemid. The resulting DNA substrate is **mutagenized** ~~mutagenzied~~ by RSR in any method known in the art, transfected into host cells, and subjected to a screen or selection for a desired property or improved phenotype. DNA from the selected or screened phagemids is amplified, by, for example, PCR or plasmid preparation. This DNA preparation contains the various mutant sequences that one wishes to permute. This DNA is fragmented and denatured, and annealed with single-stranded DNA (ssDNA) phagemid template

(ssDNA encoding the wild-type gene and vector sequences). A preferred embodiment is the use of dut(-) ung(-) host strains such as CJ236 (Sambrook et al., Molecular Cloning CSH Press (1987)) for the preparation of ssDNA.

Please replace the paragraph beginning at page 42, line 27 with the following amended paragraph:

The negative effect of rare *E. coli* codons on expression of recombinant proteins in this host has been clearly demonstrated (Rosenberg, et al., J. Bact. 175:716-722 (1993)). However, general rules for the choice of codon usage patterns to optimize expression of functional protein have been elusive. In some embodiments of the invention, protein expression is optimized by changing codons used in the gene of interest, based on the degeneracy of the genetic code. Typically, this is accomplished by synthesizing the gene using degenerate oligonucleotides. In some embodiments the degenerate oligonucleotides have the general structure of about 20 nucleotides of identity to a DNA substrate molecule encoding a protein of interest, followed by a region of about 20 degenerate nucleotides which encode a region of the protein, followed by another region of about 20 nucleotides of identity. In a preferred embodiment, the region of identity utilizes preferred codons for the host. In a further embodiment, the oligonucleotides are identical to the DNA substrate at least one 5' and one 3' nucleotide, but have at least 85% sequence homology to the DNA substrate molecule, with the difference due to the use of degenerate codons. In some embodiments, a set of such degenerate oligonucleotides is used in which each oligonucleotide overlaps with another by the general formula n - 10, wherein n is the length of the oligonucleotide. Such oligonucleotides are typically about 20 - 1000 nucleotides in length. The assembled genes are then cloned, expressed, and screened or selected for improved expression. The assembled genes can be subjected to recursive recombination methods as **described** ~~**deseibed**~~ above until the desired improvement is achieved.

Please replace the paragraph beginning at page 47, line 4 with the following amended paragraph:

In some embodiments of the invention, overexpression of a protein can lead to the accumulation of folding intermediates which have a tendency to aggregate. Without being limited to any one theory, the role of chaperonins is thought to be to stabilize such folding intermediates

against **<u>aggregation</u>** ~~aggregration~~; thus, overexpression of a protein of interest can lead to overwhelming the capacity of chaperonins. Chaperonin genes can be evolved using the techniques of the invention, either alone or in combination with the genes encoding the protein of interest, to overcome this problem.

Please replace the paragraph beginning at page 48, line 30 with the following amended paragraph:

Many overexpressed proteins of biotechnological interest are secreted into the periplasm or media to give advantages in purification or activity assays. Optimization for high level secretion is difficult because the process is controlled by many genes and hence optimization may require multiple mutations affecting the expression level and structure of several of these components. Protein secretion in *E. coli*, for example, is known to be influenced by many proteins including: a secretory ATPase (SecA), a translocase complex (SecB, SecD, SecE, SecF, and SecY), chaperonins (DnaK, DnaJ, GroES, GroEL), signal peptidases (LepB, LspA, Ppp), specific folding catalysts (DsbA) and other proteins of less well defined function (e.g., Ffh, FtsY) (Sandkvist et al., <u>Curr. Op. Biotechnol.</u> 7:505-511 (1996)). Overproduction of wild type or mutant copies of these genes for these proteins can significantly increase the yield of mature secreted protein. For example, overexpression of secY or secY4 significantly increased the periplasmic yield of mature human IL6 from a hIL6-pre-OmpA fusion (Perez-Perez et al., <u>Bio-Technology</u> 12:178-180 (1994)). Analogously, overexpression of DnaK/DnaJ in *E. coli* improved the yield of secreted human granulocyte colony stimulating factor (Perez-Perez et al., <u>Biochem. Biophys. Res. Commun.</u> **<u>210:524-529</u>** ~~210:254-259~~ (1995)).

Please replace the paragraph beginning at page 51, line 1 with the following amended paragraph:

For example, an efficiently secreted thermostable DNA polymerase can be evolved, thus allowing the performance of DNA polymerization assays with little or no purification of the expressed DNA polymerase. Such a procedure would be preferred for the expression of libraries of mutants of any protein that one wished to test in a high throughput assay, for example any of the pharmaceutical proteins listed in Table I, or any industrial enzyme. Initial constructs are made by fusing a signal peptide such as that from STII or OmpA to the amino terminus of the protein to be

secreted. A gene cluster of cloned genes believed to act in the secretory pathway of interest are mutagenized and coexpressed with the target construct. Individual clones are screened for **expression** ~~expresion~~ of the gene product. The secretory gene clusters from improved clones are recovered and recloned and introduced back into the original host. Preferably, they are first subjected to mutagenesis before the process is repeated. This cycle is repeated until the desired improvement in expression of secreted protein is achieved.

Please replace the paragraph beginning at page 51, line 28 with the following amended paragraph:

The application of evolutionary technologies to industrial enzymes is often significantly limited by the types of selections that can be applied and the modest numbers of mutants that can be surveyed in screens. Selection of enzymes or catalytic antibodies, expressed in a display format, for binding to transition state analogs (McCafferty et al., Appl. Biochem. Biotechnol. **47:157-173** ~~47:157-171~~ (1994)) or substrate analogs (Janda et al., Proc. Natl. Acad. Sci. (U.S.A.) 91:2532-2536, (1994)) represents a general strategy for selecting for mutants with ~~with~~ improved catalytic efficiency.

Please replace the paragraph beginning at page 55, line 29 with the following amended paragraph:

Thus, in some embodiments of the invention, libraries of mutant polymerase genes are screened by direct high throughput screening for improved sequencing properties. The best candidates are then subjected to RSR. Briefly, mutant libraries of candidate polymerases such as Taq polymerase are constructed using standard methods such as PCR mutagenesis (**Cadwell** ~~Caldwell~~ et al., PCR Meth. App. 2:28-33 (1992)) and/or cassette mutagenesis (Sambrook et al., Molecular Cloning, CSH Press (1987)). Incorporation of mutations into Taq DNA polymerase such as the active site residue from T7 polymerase that improves acceptance of dideoxy nucleotides (Tabor and Richardson, J. Biol. Chem. 265:8322-8328 (1990)) and mutations that inactivate the 5' - 3' exonuclease activity (R.S. **Ranu** ~~Rano~~, BioTechniques 18:390-396 (1995)) are incorporated into these libraries. The reassembly PCR technique, for example, as described above is especially suitable for this problem. Similarly, chimeric polymerase libraries are made by breeding existing thermophilic polymerases, sequenase, and *E. coli* polI with each other using the bridge

oligonucleotide methods described above. The libraries are expressed in formats wherein human or robotic colony picking is used to replica pick individual colonies into 96 well plates where small cultures are grown, and polymerase expression is induced.

Please replace the paragraph beginning at page 56, line 20 with the following amended paragraph:

A high throughput sequencing assay is used to perform sequencing reactions with the purified samples. The data is analyzed to identify mutants with improved sequencing properties, according to any of these criteria: higher quality ladders on GC-rich templates, especially greater than 60% GC, including such points as fewer artifactual termination products and stronger signals than given with the wild-type enzyme; less termination of reactions by inosine in primer labelled reactions, e.g., fluorescent labelled primers; less variation in incorporation of signals in reactions with fluorescent dideoxy nucleotides at any given position; longer sequencing ladders than obtained with the wild-type enzyme, such as about 20 to 100 nucleotides; improved acceptance of other known base analogs such as 7-deaza purines; improved acceptance of new base analogs from combinatorial chemistry libraries (See, for example, Hogan, Nature 384(Supp):**17 (1996)** ~~**17-1996**~~).

Please replace the paragraph beginning at page 59, line 1 with the following amended paragraph:

In some embodiments, GFP containing stop codons instead of lacZ alpha with stop codons is used for the construction. Cells with reverted stop codons in GFP are selected by fluorescence activated cell sorter (FACS). In general, FACS selection is performed by gating the brightest about 0.1- 10%, preferably the top 0.1 to 1%, and collected according to a protocol similar to that of Dangl et al., (Cytometry 2(6):395-401 (1982)). In other embodiments, the polA gene is flanked with lox sites or other targets of a site specific recombinase. The recombinase is induced, thus allowing one to inducibly delete the polA gene (**Medberry** ~~**Mulbery**~~ et al., Nucleic Acid Res. 23:485-490 (1995)). This would allow one to perform "Loeb-type" selections at any temperature and in any host. For example, one could set up such a selection in a recA deficient mesophile or thermophile by placing the polA homologue in an inducibly deletable format and thus apply the selection for active polymerase under more general conditions.

Please replace the paragraph beginning at page 62, line 16 with the following amended paragraph:

In a further embodiment, the protease is not necessarily provided in a display format. The host cells secrete the protease encoded by but not surface **displayed** ~~**diplayed**~~ by a phagemid, while constrained to a well, for example, in a microtiter plate. Phage display format is preferred where an entire high titre lysate is encased in a protein net matrix, and the phage expressing active and broad specificity proteases digesting the matrix to be liberated for the next round of amplification, mutagenesis, and selection.

Please replace the paragraph beginning at page 62, line 34 with the following amended paragraph:

Peptide substrates containing fluoropores attached to the carboxy terminus and fluorescence quenching **moieties** ~~**moities**~~ on the amino terminus, such as those described by Holskin, et al, (Anal. Biochem. **226** ~~227~~:148-55 (1995)) (e.g., (4-4'-dimethylaminophenazo)benzoyl-arg-gly-val-val-asn-ala-ser-ser-arg-leu-ala-5-(2'-aminoethyl)-amino]-naphthalene-1-sulfonic acid) **(SEQ ID NO:99)** are used to screen protease mutants for broadened or altered specificity. In brief, a library of peptide substrates is designed with a **fluorophore** ~~**flourophore**~~ on the amino terminus and a potent fluorescence quencher on the carboxy terminus, or vice versa. Supernatants containing secreted proteases are incubated either separately with various members of the library or with a complex cocktail. Those proteases which are highly active and have broad specificity will cleave the majority of the peptides, thus releasing the fluorophore from the quencher and giving a positive signal on a fluorimeter. This technique is amenable to a high density multiwell format.

Please replace the paragraph beginning at page 64, line 22 with the following amended paragraph:

In some embodiments, the following method can be used for selection. A lysate of phage encoding IFN alpha mutants, for example, can be used directly at suitable dilution to stimulate cells with a GFP reporter construct (Crameri et al., **Nat. Biotech.** ~~**Nat. Med.**~~ 14:315-319 (1996)) under the control of an IFN responsive promoter, such as an MHC class I promoter. Phage remaining attached after stimulation, expression and FACS purification of the responsive cells, can

be purified by FACS. Preferably, the brightest cells are collected. The phage are collected and their DNA subjected to RSR until the level of desired improvement is achieved.

Please replace the paragraph beginning at page 65, line 15 with the following amended paragraph:

In some embodiments, the eukaryotic cell is the unit of biological selection. The following general protocol can be used to apply RSR to the improvement of proteins using eukaryotic cells as the unit of selection: (1) transfection of libraries of mutants into a suitable host cell, (2) expression of the encoded gene product(s) either transiently or stably, (3) functional selection for cells with an improved phenotype (expression of a receptor with improved affinity for a target ligand; viral resistance, etc., (4) recovery of the mutant genes by, for example, PCR followed by preparation of HIRT supernatants with subsequent **transformation** ~~**tranformation**~~ of *E. coli*, (5) RSR and (6) repetition of steps (1) - (5) until the desired degree of improvement is achieved.

Please replace the paragraph beginning at page 66, line 16 with the following amended paragraph:

The use of a one mutant sequence-one transfected cell protocol is a preferred design feature for RSR based protocols because the point is to use functional selection to identify mutants with improved phenotypes and, if the transfection is not done in a "clonal" fashion, the functional phenotype of any given cell is the result of the sum of many transfected sequences. Protoplast fusion is one method to achieve this end, since each protoplast contains typically greater than 50 copies each of a single plasmid variant. However, it is a relatively low efficiency process (about 10^3 - 10^4 transfectants), and it does not work well on some non-adherent cell lines such as B cell lines. Retroviral vectors provide a second alternative, but they are limited in the size of acceptable insert (<10 kb) and consistent, high expression levels are sometimes difficult to achieve. Random integration results in varying expression levels, thus introducing noise and limiting one's ability to distinguish between improvements in the affinity of the mutant protein vs. increased expression. A related class of strategies that can be used effectively to achieve "one gene-one cell" DNA transfer and consistent expression levels for RSR is to use a viral vector which contains a lox site and to introduce this into a host that expresses cre recombinase, preferably transiently, and contains one or

more lox sites integrated into its genome, thus limiting the variability of integration sites (**Rohlmann** ~~Rohlman~~ et al. Nature Biotech. 14:1562-1565 (1996)).

Please replace the paragraph beginning at page 67, line 37 with the following amended paragraph:

In some embodiments, mutant proteins are selected or screened based on their ability to exert a biological effect in an autocrine fashion on the cell expressing the mutant protein. For example, a library of alpha interferon genes can be selected for induction of more potent or more specific antiviral activity as follows. A library of interferon alpha mutants is generated in a vector which allows for induction of expression (i.e. under control of a metallothionein promoter) and efficient secretion in a multiwell format (96-well for example) with one or a few independent clones per well. In some embodiments, the promoter is not **inducible, and** ~~inducible,and~~ may be constitutive.

Please replace the paragraph beginning at page 71, line 24 with the following amended paragraph:

In further embodiments of the invention, the protein of interest is evolved to have increased shelf life. A library of the mutagenized nucleic acid **sequence** ~~squence~~ encoding the protein of interest is expressed in a display format or high throughput expression format, and exposed for various lengths of time to conditions for which one wants to evolve stability (heat, metal ions, nonphysiological pH of, for example, <6 or >8, lyophilization, freeze-thawing). Genes are recovered from ~~from~~ survivors, for example, by PCR. The DNA is subjected to mutagenesis, such as RSR, and the process repeated until the desired level of improvement is achieved.

Please replace the paragraph beginning at page 71, line 37 with the following amended paragraph:

As discussed above, in some embodiments of the invention, the substrate for evolution by RSR is preferably a single chain **construction** ~~contruction~~. The possibility of performing **asymmetric** ~~asymetric~~ mutagenesis on constructs of homomultimeric proteins provides important new pathways for further evolution of such constructs that is not open to the proteins in their natural homomultimeric states. In particular, a given mutation in a homomultimer will result in

that change being present in each identical subunit. In single chain constructs, however, the domains can mutate independently of each other.

Please replace the paragraph beginning at page 72, line 10 with the following amended paragraph:

Conversion of multisubunit proteins to single chain constructs with new and useful properties has been demonstrated for a number of proteins. Most notably, antibody heavy and light chain variable domains have been linked into single chain Fv's (Bird et al., Science 242:423-426 (1988)), and this strategy has resulted in antibodies with improved thermal stability (Young et al., FEBS Lett 377:135-139 (1995)), or sensitivity to proteolysis (Solar et al., Prot. Eng. 8:717-723 (1995)). A functional single chain version of IL5, a homodimer, has been constructed, shown to have affinity for the IL5 receptor similar to that of wild type protein, and this construct has been used to perform **asymmetric** ~~**assymetric**~~ mutagenesis of the dimer (Li et al., J. Biol. Chem. 271:1817-1820 (1996)). A single chain version of urokinase-type plasminogen activator has been made, and it has been shown that the single chain construct is more resistant to plasminogen activator inhibitor type 1 than the native homodimer (Higazi et al., Blood 87:3545-3549 (1996)). Finally, a single-chain insulin-like growth factor I/insulin hybrid has been constructed and shown to have higher affinity for chimeric insulin/IGF-1 receptors than that of either natural ligand (Kristensen et al., Biochem. J. 305:981-986 (1995)).

Please replace the paragraph beginning at page 72, line 31 with the following amended paragraph:

In general, a linker is constructed which joins the amino terminus of one subunit of a protein of interest to the carboxyl terminus of another subunit in the complex. These fusion proteins can consist of linked versions of homodimers, homomultimers, heterodimers or higher order heteromultimers. In the simplest case, one adds polypeptide linkers between the native termini to be joined. Two significant variations can be made. First, one can construct diverse libraries of variations of the wild type sequence in and around the junctions and in the linkers to facilitate the construction of active fusion proteins. Secondly, Zhang et al., (Biochemistry 32:12311-12318 (1993)) have described circular permutations of T4 lysozyme in which the native amino and carboxyl termini have been joined and novel amino and carboxyl termini have been engineered into

the protein. The methods of circular permutation, libraries of linkers, and libraries of junctional sequences flanking the linkers allow one to construct libraries that are diverse in topological linkage strategies and in primary sequence. These libraries are expressed and selected for activity. Any of the above mentioned strategies for screening or selection can be used, with phage display being preferable in most cases. Genes encoding active fusion proteins are recovered, mutagenized, reselected, and subjected to standard RSR protocols to optimize their function. Preferably, a population of selected mutant single chain constructs is PCR amplified in two **separate** ~~seprate~~ PCR reactions such that each of the two domains is amplified separately. Oligonucleotides are derived from the 5' and 3' ends of the gene and from both strands of the linker. The separately amplified domains are shuffled in separate reactions, then the two populations are recombined using PCR reassembly to generate intact single chain constructs for further rounds of selection and evolution.

Please replace the paragraph beginning at page 77, line 6 with the following amended paragraph:

Using the screens and selections listed above, RSR can be used in several ways to modify eukaryotic signal transduction or transcriptional pathways. Any component of a signal transduction pathway of interest, of the regulatory regions and transcriptional activators that interact with this region and with chemicals that induce transcription can be evolved. This generates regulatory systems in which transcription is activated more potently by the natural inducer or by analogues of the normal inducer. This technology is preferred for the development and optimization of diverse assays of biotechnological interest. For example, dozens of 7 transmembrane receptors (7-TM) are validated targets for drug discovery (see, for example, Siderovski et al., Curr Biol., 6(2):211-212 (1996); An et al., FEBS Lett., 375(1-2):121-124 (1995); Raport et al., Gene, 163(2):295-299 (1995); Song et al., Genomics, 28(2):347-349 (1995); Strader et al. FASEB J., 9(9):745-754 (1995); Benka et al., FEBS Lett., 363(1-2):49-52 (1995); Spiegel, J. Clin Endocrinol. Metab., 81(7):2434-2442 (1996); Post et al., FASEB J., 10(7):741-749 (1996); Reisine et al., Ann NY Acad. Sci., 780:168-175 (1996); Spiegel, Annu. **Rev.** ~~Ref.~~ Physiol., 58:143-170 **(1995)** ~~(1996)~~; Barak et al., Biochemistry, 34(47):15407-15414 (1995); and Shenker, Baillieres Clin. Endocrinol. Metab., 9(3):427-451 (1995)). The development of sensitive high throughput assays for agonists and antagonists of these receptors is essential for exploiting the full potential of combinatorial chemistry in discovering such ligands. Additionally, biodetectors or biosensors for different chemicals can be

developed by evolving 7-TM's to respond agonistically to novel chemicals or proteins of interest. In this case, selection would be for **constructs** ~~contructs~~ that are activated by the new chemical or polypeptide to be detected. Screening could be done simply with fluorescence or light activated cell sorting, since the desired improvement is coupled to light production.

Please replace the paragraph beginning at page 79, line 33 with the following amended paragraph:

The methods described herein can be used to evolve the coupling of mammalian 7-TM receptors to yeast signal transduction pathways. A typical approach is as follows: (1) clone a 7-TM of interest into a yeast strain with a modified pheromone response pathway similar to that described by Price (e.g., strains deficient in FAR1, a negative regulator of G_1 cyclins, and deficient in SST2 which causes the cells to be hypersensitive to the presence of pheromone), (2) construct libraries of chimeras between the mammalian G alpha protein(s) known or thought to interact with the GPA1 or homologous yeast G alpha proteins, (3) place a selectable reporter gene such as HIS3 under control of the pheromone responsive promoter FUS1 (Price et al., Mol. Cell Biol. 15:6188-6195 (1995)). Alternatively, a screenable gene such as luciferase may be placed under the control of the FUS1 promoter; (4) transform library (2) into strain (3) (HIS(-)), (5) screen or select for expression of the reporter in response to the ligand of interest, for example by growing the library of transformants on minimal plates in the presence of ligand to demand HIS3 expression, (6) recover the selected cells, and ~~and~~ apply RSR to evolve improved expression of the reporter under the control of the pheromone responsive promoter FUS1.

Please replace the paragraph beginning at page 82, line 17 with the following amended paragraph:

A preferred strategy to evolve BIAP is as follows. A codon usage **library** ~~libary~~ is constructed from 60-mer oligonucleotides such that the central 20 bases of each oligo specifies the wild type protein, but encodes the wild-type protein sequence with degenerate codons. Preferably, very rare codons for the prokaryotic host of choice, such as *E. coli*, are not used. The 20 bases at each end of the oligo use non-degenerate, but preferred, codons in *E. coli*. The oligonucleotides are assembled into full-length genes as described above. The assembled products are cloned into an expression vector by techniques well known in the art. In some embodiments, the codon usage

library is expressed with a library of secretory leader sequences, each of which directs the encoded BIAP protein to the *E. coli* periplasm. A library of leader sequences is used to optimize the combination of leader sequence and mutant. Examples of leader sequences are reviewed by Schatz et al. (Ann Rev. Genet. 24:215-248 (1990)). The cloned BIAP genes are expressed under the control of an inducible promoter such as the arabinose promoter. Arabinose- induced colonies are screened by spraying with a substrate for BIAP, bromo-chloro-indolyl phosphate (BCIP). The bluest colonies are picked visually and subjected to the RSR procedures described herein.

Please replace the paragraph (Table II) beginning at page 83, line 6 and ending at page 86, line 1, with the following amended paragraph:

Table II

1. AACCCTCCAG TTCCGAACCC CATATGATGA TCACCCTGCG TAAACTGCCG **(SEQ ID NO:1)**
2. AACCCTCCAG TTCCGAACCC CATATGAAAA AAACCGCT **(SEQ ID NO:2)**
3. AACCCTCCAG TTCCGAACCC ATATACATAT GCGTGCTAAA **(SEQ ID NO:3)**
4. AACCCTCCAG TTCCGAACCC CATATGAAAT ACCTGCTGCC GACC **(SEQ ID NO:4)**
5. AACCCTCCAG TTCCGAACCC GATATACATA TGAAACAGTC **(SEQ ID NO:5)**
6. TGGTGTTATG TCTGCTCAGG CDATGGCDGT DGAYTTYCAY CTGGTTCCGG TTGAAGAGGA **(SEQ ID NO:6)**
7. GGCTGGTTTC GCTACCGTTG CDCARGCDGC DCCDAARGAY CTGGTTCCGG TTGAAGAGGA **(SEQ ID NO:7)**
8. CACCCCGATC GCTATCTCTT CYTTYGCDTC YACYGGYTCY CTGGTTCCGG TTGAAGAGGA **(SEQ ID NO:8)**
9. GCTGCTGGCT GCTCAGCCGG CDATGGCDAT GGAYATYGGY CTGGTTCCGG TTGAAGAGGA **(SEQ ID NO:9)**
10. TGCCGCTGCT GTTCACCCCG GTDACYAARG CDGCDCARGT DCTGGTTCCG GTTGAAGAGG A **(SEQ ID NO:10)**
11. CCCGGCTTTC TGGAACCGTC ARGCDGCDCA RGCDCTGGAC GTTGCTAAAA AACTGCAGCC **(SEQ ID NO:11)**
12. ACGTTATCCT GTTCCTGGGT GAYGGYATGG GYGTDCCDAC CGTTACCGCT ACCCGTATCC **(SEQ ID NO:12)**

13. AAACTGGGTC CGGAAACCCC DCTGGCDATG GAYCARTTYC CGTACGTTGC TCTGTCTAAA **(SEQ ID NO:13)**
14. GGTTCCGGAC TCTGCTGGTA CYGCDACYGC DTAYCTGTGC GGTGTTAAAG GTAACTACCG **(SEQ ID NO:14)**
15. CTGCTCGTTA CAACCAGTGC AARACYACYC GYGGYAAYGA AGTTACCTCT GTTATGAACC **(SEQ ID NO:15)**
16. TCTGTTGGTG TTGTTACCAC YACYCGYGTD CARCAYGCDT CTCCGGCTGG TGCTTACGCT **(SEQ ID NO:16)**
17. GTACTCTGAC GCTGACCTGC CDGCDGAYGC DCARATGAAC GGTTGCCAGG ACATCGCTGC **(SEQ ID NO:17)**
18. ACATCGACGT TATCCTGGGT GGYGGYCGYA ARTAYATGTT CCCGGTTGGT ACCCCGGACC **(SEQ ID NO:18)**
19. TCTGTTAACG GTGTTCGTAA RCGYAARCAR AAYCTGGTDC AGGCTTGGCA GGCTAAACAC **(SEQ ID NO:19)**
20. GAACCGTACC GCTCTGCTGC ARGCDGCDGA YGAYTCYTCT GTTACCCACC TGATGGGTCT **(SEQ ID NO:20)**
21. AATACAACGT TCAGCAGGAC CAYACYAARG AYCCDACYCT GCAGGAAATG ACCGAAGTTG **(SEQ ID NO:21)**
22. AACCCGCGTG GTTTCTACCT GTTYGTDGAR GGYGGYCGYA TCGACCACGG TCACCACGAC **(SEQ ID NO:22)**
23. GACCGAAGCT GGTATGTTCG AYAAYGCDAT YGCDAARGCT AACGAACTGA CCTCTGAACT **(SEQ ID NO:23)**
24. CCGCTGACCA CTCTCACGTT TTYTCYTTYG GYGGYTAYAC CCTGCGTGGT ACCTCTATCT **(SEQ ID NO:24)**
25. GCTCTGGACT CTAAATCTTA YACYTCYATY CTGTAYGGYA ACGGTCCGGG TTACGCTCTG **(SEQ ID NO:25)**
26. CGTTAACGAC TCTACCTCTG ARGAYCCDTC YTAYCARCAG CAGGCTGCTG TTCCGCAGGC **(SEQ ID NO:26)**
27. AAGACGTTGC TGTTTTCGCT CGYGGYCCDC ARGCDCAYCT GGTTCACGGT GTTGAAGAAG **(SEQ ID NO:27)**
28. ATGGCTTTCG CTGGTTGCGT DGARCCDTAY ACYGAYTGYA ACCTGCCGGC TCCGACCACC **(SEQ ID NO:28)**
29. TGCTCACCTG GCTGCTTMAC CDCCDCCDCT GGCDCTGCTG GCTGGTGCTA TGCTGCTCCT C **(SEQ ID NO:29)**

30. TTCCGCCTCT AGAGAATTCT TARTACAGRG THGGHGCCAG GAGGAGCAGC ATAGCACCAG CC (SEQ ID NO:30)
31. AAGCAGCCAG GTGAGCAGCG TCHGGRATRG ARGTHGCGGT GGTCGGAGCC GGCAGGTT (SEQ ID NO:31)
32. CGCAACCAGC GAAAGCCATG ATRTGHGCHA CRAARGTYTC TTCTTCAACA CCGTGAACCA (SEQ ID NO:32)
33. GCGAAAACAG CAACGTCTTC RCCRCCRTGR GTYTCRGAHG CCTGCGGAAC AGCAGCCTGC (SEQ ID NO:33)
34. AGAGGTAGAG TCGTTAACGT CHGGRCGRGA RCCRCCRCCC AGAGCGTAAC CCGGACCGTT (SEQ ID NO:34)
35. AAGATTTAGA GTCCAGAGCT TTRGAHGGHG CCAGRCCRAA GATAGAGGTA CCACGCAGGG (SEQ ID NO:35)
36. ACGTGAGAGT GGTCAGCGGT HACCAGRATC AGRGTRTCCA GTTCAGAGGT CAGTTCGTTA (SEQ ID NO:36)
37. GAACATACCA GCTTCGGTCA GHGCCATRTA HGCYTTRTCG TCGTGGTGAC CGTGGTCGAT (SEQ ID NO:37)
38. GGTAGAAACC ACGCGGGTTA CGRGAHACHA CRCGCAGHGC AACTTCGGTC ATTTCCTGCA (SEQ ID NO:38)
39. TCCTGCTGAA CGTTGTATTT CATRTCHGCH GGYTCRAACA GACCCATCAG GTGGGTAACA (SEQ ID NO:39)
40. CAGCAGAGCG GTACGGTTCC AHACRTAYTG HGCRCCYTGG TGTTTAGCCT GCCAAGCCTG (SEQ ID NO:40)
41. TACGAACACC GTTAACAGAA GCRTCRTCHG GRTAYTCHGG GTCCGGGGTA CCAACCGGGA (SEQ ID NO:41)
42. CCCAGGATAA CGTCGATGTC CATRTTRTTH ACCAGYTGHG CAGCGATGTC CTGGCAACCG (SEQ ID NO:42)
43. CAGGTCAGCG TCAGAGTACC ARTTRCGRTT HACRGTRTGA GCGTAAGCAC CAGCCGGAGA (SEQ ID NO:43)
44. TGGTAACAAC ACCAACAGAT TTRCCHGCYT TYTTHGCRCG GTTCATAACA GAGGTAACTT (SEQ ID NO:44)
45. CACTGGTTGT AACGAGCAGC HGCRGAHACR CCRATRGTRC GGTAGTTACC TTTAACACCG (SEQ ID NO:45)
46. ACCAGCAGAG TCCGGAACCT GRCGRTCHAC RTTRTARGTT TTAGACAGAG CAACGTACGG (SEQ ID NO:46)

47. GGGTTTCCGG ACCCAGTTTA CCRTTCATYT GRCCYTTCAG GATACGGGTA GCGGTAACGG **(SEQ ID NO:47)**
48. CCCAGGAACA GGATAACGTT YTTHGCHGCR GTYTGRATHG GCTGCAGTTT TTTAGCAACG **(SEQ ID NO:48)**
49. ACGGTTCCAG AAAGCCGGGT CTTCCTCTTC AACCGGAACC AG **(SEQ ID NO:49)**
50. CCTGAGCAGA CATAACACCA GCHGCHACHG CHACHGCCAG CGGCAGTTTA CGCAGGGTGA **(SEQ ID NO:50)**
51. ACCGGGGTGA ACAGCAGCGG CAGCAGHGCC AGHGCRATRG TRGACTGTTT CATATGTATA TC **(SEQ ID NO:51)**
52. GCCGGCTGAG CAGCCAGCAG CAGCAGRCCH GCHGCHGCGG TCGGCAGCAG GTAGTTTCA **(SEQ ID NO:52)**
53. AAGAGATAGC GATCGGGGTG GTCAGHACRA TRCCCAGCAG TTTAGCACGC ATATGTATAT **(SEQ ID NO:53)**
54. CAACGGTAGC GAAACCAGCC AGHGCHACHG CRATHGCRAT AGCGGTTTTT TTCATATG **(SEQ ID NO:54)**
55. AGAATTCTCT AGAGGCGGAA ACTCTCCAAC TCCCAGGTT **(SEQ ID NO:55)**
56. TGAGAGGTTG AGGGTCCAAT TGGGAGGTCA AGGCTTGGG **(SEQ ID NO:56)**

Please replace the paragraph beginning at page 86, line 24 with the following amended paragraph:

Genomic antibody expression shuttle vectors similar to those described by Gascoigne et al. (Proc. Natl. Acad. Sci. (U.S.A.) 84:2936-2940 (1987)) are constructed such that libraries of mutant V region exons can be readily cloned into the shuttle vectors. The kappa construct is cloned onto a plasmid encoding puromycin resistance and the heavy chain is cloned onto a neomycin resistance encoding vector. The cDNA derived variable region sequences encoding the mature and germline heavy and light chain V regions are reconfigured by PCR mutagenesis into genomic exons flanked by Sfi I sites with complementary Sfi I sites placed at the appropriate locations in the genomic shuttle vectors. The oligonucleotides used to create the intronic Sfi I sites flanking the VDJ exon are: 5' Sfi I: 5'-TTCCATTTCA TACATGGCCG AAGGGGCCGT GCCATGAGGA TTTT-3' **(SEQ ID NO:100)**; 3' Sfi I: 5'-TTCTAAATG CATGTTGGCC TCCTTGGCCG GATTCTGAGC CTTCAGGACC A-3' **(SEQ ID NO:101)**. Standard PCR mutagenesis protocols are applied to produce libraries of mutants wherein the following sets of residues (numbered according to Kabat,

Sequences of Proteins of Immunological Interest, U.S. Dept of Health and Human Services, 1991) are randomized to NNK codons (GATC,GATC,GC):

Please replace the paragraph beginning at page 87, line 27 with the following amended paragraph:

The p-nitrophenylphosphonate hapten (JWJ-1) recognized by this antibody is synthesized as described by Patten et al. (Science 271:1086-1091 (1996)). JWJ-1 is coupled directly to 5-(((2-aminoethyl)thio)acetyl)fluorescein (Molecular Probes, Inc.) by formation of an amide bond using a standard coupling chemistry such as EDAC (March, Advanced Organic Chemistry, Third edition, John Wiley and Sons, 1985) to give a monomeric JWJ-1-FITC probe. A "dimeric" conjugate (two molecules of JWJ-1 coupled to a FACS marker) is made in order to get a higher avidity probe, thus making low affinity interactions (such as with the germline antibody) more readily detected by FACS. This is generated by staining with Texas Red conjugated to an anti-fluorescein antibody in the presence of two equivalents of JWJ-1-FITC. The bivalent structure of IgG then provides a homogeneous bivalent reagent. A spin column is used to remove excess JWJ-1-FITC molecules that are not bound to the anti-FITC reagent. A tetravalent reagent is made as follows. One equivalent of biotin is coupled with EDAC to two equivalents of ethylenediamine, and this is then ~~be~~ coupled to the free carboxylate on JWJ-1. The **biotinylated** ~~biotiylated~~ JWJ-1 product is purified by ion exchange chromatography and characterized by mass spectrometry. FITC labelled avidin is incubated with the biotinylated JWJ-1 in order to generate a tetravalent probe.

Please replace the paragraph beginning at page 94, line 3 with the following amended paragraph:

In brief, each of the nine segments is synthesized with one degenerate oligo per segment. Degeneracies are chosen to capture all of the IFN-alpha diversity that can be captured with a single degenerate codon without adding any non-natural sequence. A second set of degenerate oligonucleotides encoding the nine segments is generated wherein all of the natural diversity is captured, but additional non-natural mutations are included at positions where necessitated by the constraints of the genetic code. In most cases all of the diversity can be captured with a single degenerate codon; in some cases a degenerate codon will capture all of the natural diversity but will add one non-natural mutation; at a few **positions** ~~postions~~ it is not possible to capture the natural

diversity without putting in a highly degenerate codon which will create more than one non-natural mutation. It is at these positions that this second set of oligonucleotides will differ from the first set by being more inclusive. Each of the nine synthetic segments is then amplified by PCR with the 18 PCR oligonucleotides. Full length genes using the oligo directed recombination method are generated, transfected into a host, and assayed for hybrids with desired properties. The best hybrids from (**e.g.,** ~~e.g,~~ the top 10%, 1% or 0.1%; preferably the top 1%) are subjected to RSR and the process repeated until a candidate with the desired properties is obtained.

Please replace the paragraph beginning at page 98, line 28 with the following amended paragraph:

A sample protocol follows for the autocrine display of IFN alpha mutants. In brief, a library of IFN mutants is generated in a vector which allows for induction of expression (i.e. metallothionein promoter) and efficient secretion. The recipient cell line carrying an IFN responsive reporter cassette [GFP or luciferase] is induced by transfection with the mutant IFN constructs. Mutants which stimulate the IFN responsive promoter are detected by ~~by~~ FACS or CCD camera.

Please replace the paragraph (Table IV) beginning at page 99, line 7 and ending at page 99, line 27, with the following amended paragraph:

Table IV

Oligonucleotides needed for blockwise recombination: 18

Oligonucleotides for alpha interferon shuffling

1. 5'-TGT[G/A]ATCTG[C/T]CT[C/G]AGACC **(SEQ ID NO:57)**
2. 5'-GGCACAAATG[G/A/C]G[A/C]AGAATCTCTC **(SEQ ID NO:58)**
3. 5'-AGAGATTCT[G/T]C[C/T/G]CATTTGTGCC **(SEQ ID NO:59)**
4. 5'-CAGTTCCAGAAG[A/G]CT[G/C][C/A]AGCCATC **(SEQ ID NO:60)**
5. 5'-GATGGCT[T/G][G/C]AG[T/C]CTTCTGGAACTG **(SEQ ID NO:61)**
6. 5'-CTTCAATCTCTTCA[G/C]CACA **(SEQ ID NO:62)**
7. 5'-TGTG[G/C]TGAAGAGATTGAAG **(SEQ ID NO:63)**
8. 5'-GGA[T/A][G/C]AGA[C/G][C/G]CTCCTAGA **(SEQ ID NO:64)**
9. 5'-TCTAGGAG[G/C][G/C]TCT[G/C][T/A]TCC **(SEQ ID NO:65)**
10. 5'-GAACTT[T/G/A][T/A]CCAGCAA[A/C]TGAAT **(SEQ ID NO:66)**
11. 5'-ATTCA[T/G]TTGCTGG[A/T][A/T/C]AAGTTC **(SEQ ID NO:67)**

12. 5'-GGACT[T/C]CATCCTGGCTGTG **(SEQ ID NO:68)**
13. 5'-CACAGCCAGGATG[G/A]AGTCC **(SEQ ID NO:69)**
14. 5'-AAGAATCACTCTTTATCT **(SEQ ID NO:70)**
15. 5'-AGATAAAGAGTGATTCTT **(SEQ ID NO:71)**
16. 5'-TGGGAGGTTGTCAGAGCAG **(SEQ ID NO:72)**
17. 5'-CTGCTCTGACAACCTCCCA **(SEQ ID NO:73)**
18. 5'-TCA[A/T]TCCTT[C/A]CTC[T/C]TTAA **(SEQ ID NO:74)**

Please amend the specification to insert the new paper copy of the Sequence Listing submitted concurrently herewith.